Rejections Under 35 U.S.C. §103

The Examiner has rejected claims 43 through 52 as obvious based on the teachings of Thompson (US 5,164,301) in view of Clark et al. (WO 98/53301) and Fisher Biotechnology Catalog (1995, page 114 and 116). Specifically, Examiner Weber states that Thompson teaches antimicrobial testing (AST) in parallel with microbial identification (ID). Furthermore, Examiner Weber states that turbidity measurements are used to read AST tests that florescence is used to determine antimicrobial identity. The Examiner acknowledges that Thompson teaches a non-automated, manual method.

The Examiner further states that Clark teaches an apparatus and method for performing AST and ID on the same multi-well plate during the same measurement period in parallel. Clark's method uses absorption, turbidity and fluorescent measurements. The range of times for incubation and testing ranges from 2 minutes to 24 hours.

The Examiner has cited the Fisher catalogue that teaches using a solid microtiter plate that has clear bottoms and walls that are black or white for use with fluorescent assays.

Finally, the Examiner has stated that merely automating manual steps is not sufficient to distinguish over the prior art (M.P.E.P. 2144.04).

In prior office actions the Applicants have argued that the smart algorithm as depicted in Figure 1 resulted in a rapid, highly accurate assay that provides both rapid microbial identification and antimicrobial susceptibility testing results in a manner previously unachievable by prior art methods. In a recent telephonic interview the Applicants stressed to Examiner Weber that the teachings of Thompson where non-analogous to the present situation and does not address the problem the present invention sought to solve and therefore cannot be combined with Clark or Fisher to render the present invention obvious. During the aforementioned interview the Applicants noted that Thompson teaches an assay that could provide a rapid test result for a single sample. But, the assay system described in Thompson could not be used to accurately report both identification and susceptibility if used in a large automated batch system as disclosed in the present application.

1. Thompson does not teach a method for correcting cross-talk using a microtiter plate when performing fluorescent determinations.

Thompson does not identify the problem that the present inventors identified and therefore one of ordinary skill in the art would not seek the teachings of Thompson to solve the problem of well-to-well cross-talk. Thompson teaches a macro-scale technique. When testing a single sample, assays can be performed one-at-a-time using large scale fluorometers without risking cross-talk between wells. Fluorescent cross-talk results in spurious readings, unreliable and non-reproducible results. This problem is explained at length in the present application beginning at page 15 and proceeding through page 17, line 15 results. Therefore, because Thompson does not address the problem identified and solved by the present invention, the Applicants respectfully assert that Thompson is a non-analogous teaching that persons having ordinary skill in the art would not be motivated to combine with the other cited art when seeking to address fluorescent cross-talk problems between microtiter plate wells.

The Applicants have claimed this feature of their invention in claim 43 wherein they state:

> "reading said [reaction chambers] wells for determining said microorganism's identity at said first predetermined time by directing a fluorescent light source through said [reaction chambers] wells for determining said microorganism's identity and collecting resulting fluorescent signals;
>
> *converting said fluorescent signals into artificial fluorescent units (AFUs)* and recording said AFUs;..."
> (Emphasis added.)

Note that the Applicants discuss these novel AFUs and their usefulness in the specification beginning at page 15 and proceeding through page 17, line 15 of the present application. This claimed limitation of the presently pending claims provides a solution to the problem of cross-talk and therefore permits highly accurate and reproducible results rapidly in a microtiter format. Note that Applicants have amended claim 43 to include the limitations "rapid," "microtiter plate" and defined the microtiter

plate wells (formally referred to as reaction chambers) as having clear bottoms and side walls to more specifically define their invention.

2. Fisher does not provide teachings on using microtiter plates having well with clear bottoms and clear side walls nor the use of Artificial Fluorescent Units.

The Examiner has previous stated that the Fisher catalogues teaches a plate suitable for use in a rapid microtiter assay that combines both turbidometric and fluorometric analytical techniques. Therefore, the Examiner asserts that the limitations of Thompson can be addressed by combining Fisher with Thompson. The Applicants respectfully disagree. Fisher teaches a microtiter plate having colored, or opaque walls and surface with clear well bottoms. Furthermore, Fisher *suggests* that this may eliminate reflected fluorescent light and thus improve fluorescent assay accuracy. However, as discussed in the above-cited sections from the present application, the Applicants considered and *rejected* this solution. The present invention uses *clear plastic plates.* Clear plastic plates are known to have economical and technical benefits over the plates disclosed in the Fisher catalog. Clear plastic plates leads to more reliable tubidometric assays and provides better overall visibility to assure that all wells have been inoculated.

Finally, Fisher teaches nothing regarding AFUs nor does it suggest their value as presently claimed. Consequently, although persons having ordinary skill in the art of ID/AST assay design may consult myriad catalogues when selecting reagents and supplies, such highly skilled persons do not generally seek teachings from such catalogs and would not be motivated to combine the Fisher catalogue description of their microtiter plate with the teachings of Thompson. Even if such teachings are combined, they do not contain all of the limitations of the present claim 43.

3. Clark does not teach a method for correcting cross-talk using a microtiter plate when performing fluorescent determinations.

As stated above when discussing the Thompson reference, Clark does not teach problems commonly associated with using fluorescence to indirectly measure microbial metabolic activity. Furthermore, Clark does not teach how one having ordinary skill in

the art would resolve the problem identified by the present inventor and claimed in claim 43 of the present application. Therefore, one having ordinary skill in the art would not turn to Clark to solve the problem of fluorescent cross-talk and therefore would not be motivated to combine the teachings of Clark with the teachings of Thompson or Fisher.

4. Conclusion as to Rejections under 35 U.S.C. § 103

There is no motivation to combine the prior art to solve the problem addressed by the present inventors, or to seek the teachings of the cited references, either individually, or in combination.

The Applicants have invented, and claimed, a novel method for rapidly and accurately determining microbial identification in parallel with antimicrobial susceptibility testing. Claim 43 and its dependent claims recite a method for performing rapid parallel determinations using a microtiter plate format wherein fluorescence is used to indirectly measure microbial metabolism of substrates in order to identify the microorganism. This is done in parallel with using turbidity to measure antimicrobial susceptibility. Moreover, claim 43 incorporates the smart algorithm of Figure 1 to assure rapid, yet accurate MIC and break point values.

No combination of the cited prior art teaches either the smart algorithm or the use of Artificial Fluorescent Units to make these rapids and accurate ID/AST determinations. A person having ordinary skill in the art of microtiter-plate assay design would not seek the teachings, nor be motivated to combine the teachings of the cited prior art. The prior art cited does not discuss the problems associated with measuring fluorescence in plastic microtiter wells AND solving the problem using Artificial Fluorescent Units as disclosed and claimed in the present application.

Therefore the Applicants respectfully assert that the presently amended claims are free from the prior art and are therefore allowable as amended.

CONCLUSION

For the foregoing reasons, all claims presently on file in the subject application are in condition for immediate allowance, and such action is respectfully requested.

If it is felt for any reason that direct communication with applicants' attorney would serve to advance prosecution of this case to finality, the Examiner is invited to call the undersigned attorney at the below listed telephone number.

The Commissioner is authorized to charge any fee which may be required in connection with this Amendment to deposit account No. 50-1329.

Respectfully submitted,

Dated: 6/23/03

Louis C. Cullman
Registration No. 39,645
Attorney for Applicants

STRADLING YOCCA CARLSON & RAUTH
660 Newport Center Drive, Suite 1600
Newport Beach, CA 92660
Telephone: 949.725.4000
Facsimile: 949.725.4100

VERSION WITH MARKINGS TO SHOW CHANGES

43. (Once Amended) A method for the rapid determination of a microorganism's identity and susceptibility to an antimicrobial agent comprising:

preparing a sample to be tested;

inoculating a hybrid panel having a plurality of [reaction chambers] wells with said sample, wherein said hybrid panel comprises a modified clear plastic microtiter plate having [reaction chambers] wells, said wells having clear bottoms and clear side walls for rapidly determining said microorganism's identity using fluorescence in parallel with [reaction chambers] wells for rapidly determining said microorganism's susceptibility to at least one antimicrobial agent;

placing said inoculated hybrid panel into a device that maintains said inoculated hybrid panels at a predetermined temperature for a predetermined time;

incubating said inoculated hybrid panel in said device at said predetermined temperature for a first predetermined time;

reading said [reaction chambers] wells for determining said microorganism's identity at said first predetermined time by directing a fluorescent light source through said [reaction chambers] wells for determining said microorganism's identity and collecting resulting fluorescent signals;

converting said fluorescent signals into artificial fluorescent units (AFUs) and recording said AFUs;

analyzing said AFUs using a database to establish said microorganism's identity and retaining said identity [until said microorganism's susceptibility to said at least one antimicrobial is determined];

reading said [reaction chambers] wells for determining said microorganism's susceptibility at at least one second predetermined time by transmitting a visible light source through said [reaction cambers] wells for determining said microorganism's susceptibility to at least one antimicrobial agent., collecting said resulting visible light signals and converting said visible light signals into a turbidity reading;

analyzing said turbidity reading according to the algorithm depicted in Figure 1;

determining said susceptibility to at least one antimicrobial agent as directed by the algorithms depicted in Figure 1;

retaining said parallel determination of said antimicrobial susceptibility and said microorganism's identity such that said antimicrobial sensitivity and microorganism's identity can be rapidly reported.

46. (Once amended) The method according to claim 43 wherein said sample comprises a substantially pure culture of microorganisms suspended in inoculum water.[.]

47. (Once amended) The method according to claim 43 wherein said reading said [reaction chamber] wells for determining said microorganism's susceptibility is done manually.

48. (Once amended) The method according to claim 43 wherein said reading said [reaction chamber] wells for determining said microorganism's susceptibility is done instrumentally in an automated system.

Please add new claims 53-60 as follows:

53. (New) A rapid method for eliminating false susceptible minimum inhibitory concentrations (MIC) results for beta-lactam positive microorganisms comprising:

preparing a test sample, said test sample comprising a standardized suspension of a viable beta lactam positive microorganism dispersed in a medium;

preparing a negative control sample, said negative control sample comprising said medium without a viable beta lactam positive microorganism dispersed in said medium;

adding a volume of said test sample to a plurality of drug wells, said drug wells containing at least one diluted antimicrobial agent, to form a plurality of inoculated test wells;

adding a volume of said test sample to at least one control well, said control well having no antimicrobial agent present, to form an inoculated growth control well;

adding a volume of negative control sample to at least one said control well to form an inoculated negative control well;

mixing said inoculated drug wells, said inoculated growth control well and inoculated negative control well; wherein said inoculated drug wells, said inoculated growth control well and inoculated negative control well collectively form an MIC test plate having antimicrobial susceptibility test (AST) wells;

incubating said MIC test plate at a temperature for a first predetermined interval;

determining the absorbance for each of said AST wells at the end of said first predetermined interval;

further incubating said MIC test plate at said temperature for at least one second predetermined interval;

determining the absorbance value for each of said AST wells at the end of said at least one second predetermined interval;

calculating a susceptibility index (SI) for each of drug well and growth control well using said absorbance values;

determining the ratio of said growth control well SI to said drug well SI to establish the MIC of said beta-lactam positive microorganism to said drug;

54. (New) The method according to claim 53 wherein said method for eliminating false susceptible MIC results for beta-lactam positive microorganisms further comprises using the algorithm in Figure 1.

55. (New) The method according to claim 53 wherein said SI is calculated using the equation (D*f* – C*f*)/D*i*-C*i*) where D*f* equals a second turbidity reading for each drug well, C*f* equals a second turbidity reading for each growth control well, D*i* equals a first turbidity reading for each drug well, and C*i* equals a first turbidity reading for each growth control well.

56. New) The method according to claim 53 wherein said ratio of growth control well SI to said drug well SI is close to 1 where the microorganism is truly resistant to a beta-lactam antibiotic.

57. (New) The method according to claim 53 wherein said ratio of growth control well SI to said drug well SI is greater than or equal to 3 where the microorganism is truly sensitive to a beta-lactam antibiotic.

58. (New) The method according to claim 53 wherein said microorganism is gram positive.

59. (New) The method according to claim 53 wherein said microorganism is gram negative.

60. (New) A method for the rapid determination of a microorganism's identity and susceptibility to an antimicrobial agent comprising:

preparing a sample to be tested;

inoculating a hybrid panel having a plurality of wells with said sample, wherein said hybrid panel comprises a modified clear plastic microtiter plate having wells, said wells having clear bottoms and clear side walls for rapidly determining said microorganism's identity using fluorescence in parallel with wells for rapidly determining said microorganism's susceptibility to at least one antimicrobial agent;

placing said inoculated hybrid panel into a device that maintains said inoculated hybrid panels at a predetermined temperature for a predetermined time;

incubating said inoculated hybrid panel in said device at said predetermined temperature for a first predetermined time;

reading said wells for determining said microorganism's identity at said first predetermined time by directing a fluorescent light source through said wells for determining said microorganism's identity and collecting resulting fluorescent signals;

converting said fluorescent signals into artificial fluorescent units (AFUs) and recording said AFUs;

analyzing said AFUs using a database to establish said microorganism's identity and retaining said identity;

reading said wells for determining said microorganism's susceptibility at at least one second predetermined time by transmitting a visible light source through said wells for determining said microorganism's susceptibility to at least one antimicrobial agent., collecting said resulting visible light signals and converting said visible light signals into a turbidity reading;

analyzing said turbidity reading;

determining said susceptibility to at least one antimicrobial agent;

retaining said parallel determination of said antimicrobial susceptibility and said microorganism's identity such that said antimicrobial sensitivity and microorganism's identity can be rapidly reported.